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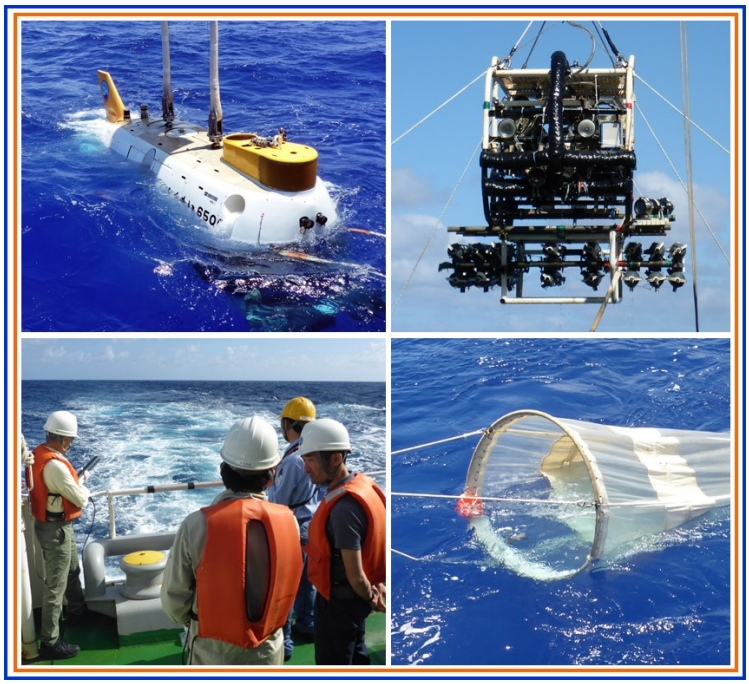
YOKOSUKA Cruise Report

YK17-10

Understanding the Spawning Ecology of Freshwater Eels



Western North Pacific along the West Mariana Ridge

13–27 MAY 2017

Japan Agency for Marine-Earth Science and Technology

(JAMSTEC)

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1. Cruise Information

● Cruise ID
YK17-10
● Name of vessel
R/V YOKOSUKA (JAMSTEC)
● Title of the cruise
Understanding the Spawning Ecology of Freshwater Eels
● Title of proposal
Understanding the Spawning Ecology of Freshwater Eels
● Cruise period
13–27 MAY 2017
● Ports of call
Yokosuka and Saipan
● Research area
Western North Pacific along the West Mariana Ridge

● Research Maps: Figure 1 (map X-CTD stations), Figure 2 (maps of YK17-10 sampling areas for each type of gear), and Figure 3 (map of internal tide patterns during YK17-10 and during previous egg collections).

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Quarter Master	KANAME HIROSAKI
Quarter Master	KAITO MURATA
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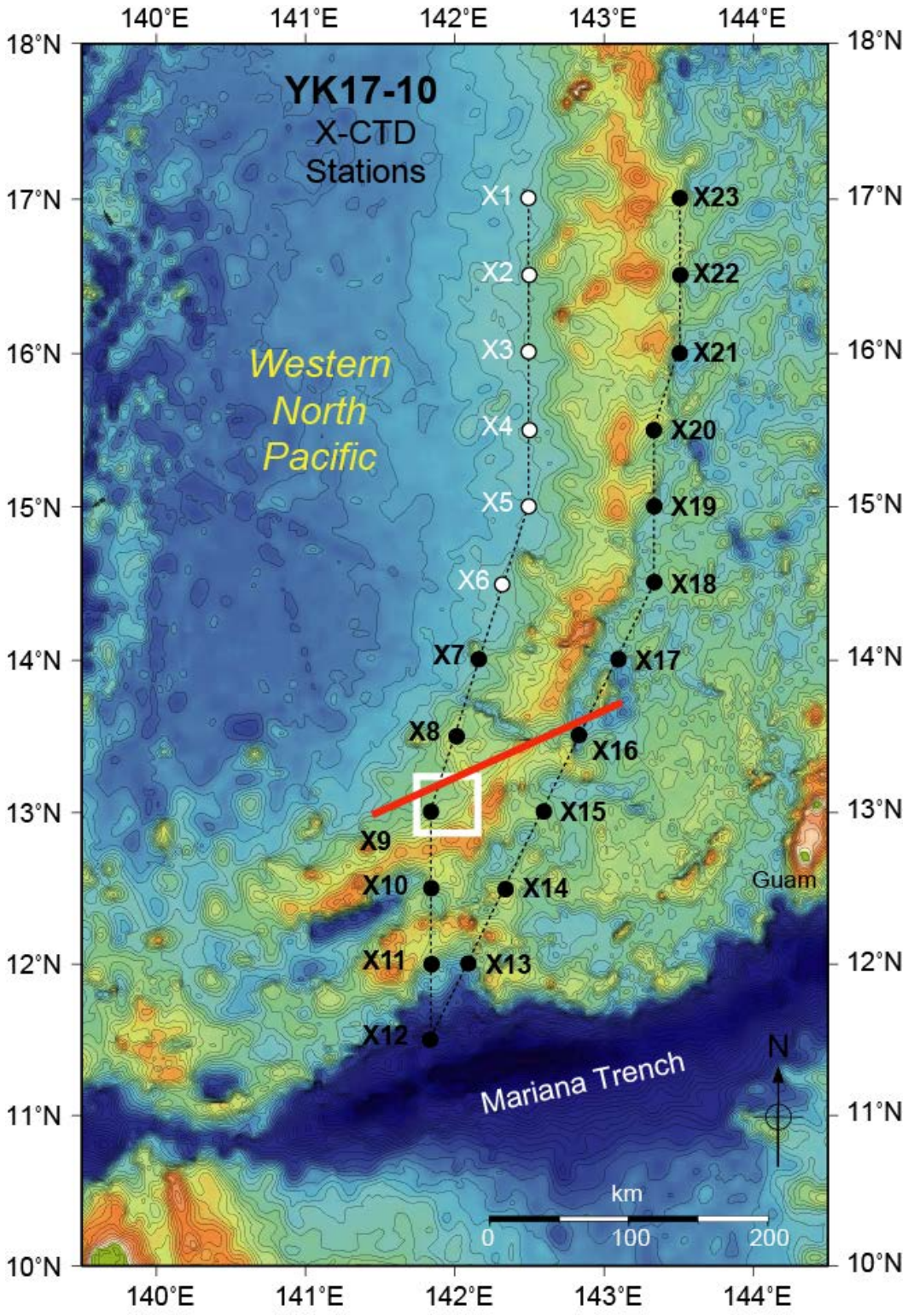

Figure 1. Map of the X-CTD stations during the YK17-10 research cruise the R/V YOKOSUKA (JAMSTEC) along the West Mariana Ridge in May 2017. The stations were sampled while moving southward along the east side of the ridge and then moving north along the west side of the ridge until a frontal feature was detected (red line), so Stn. X1–X6 were not sampled, and the observation region (white box) was defined.

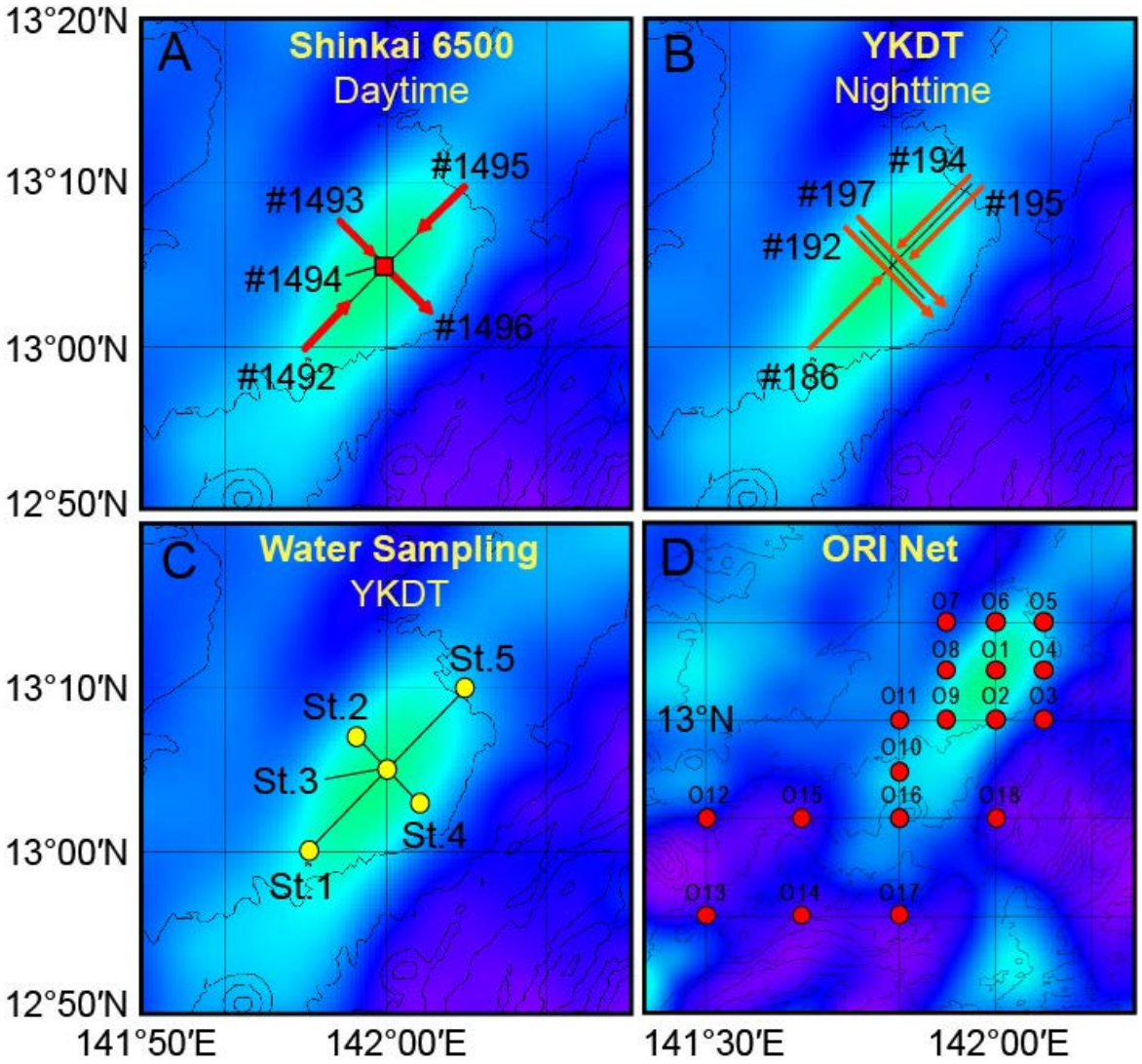

Figure 2. Maps showing the generalized deployment locations of the Shinkai 6500 submersible during 5 daytime dives (A; Figure 12) at a range of depths between 400–1000 m (Figure 13) and of the YKDT towed observation system during 5 nighttime deployments mostly at 200–250 m (B; Figure 17,18) to attempt to video record Japanese eels, the locations where the YKDT was deployed for CTD observations and to collect water samples during the day for environmental DNA analyses (C; Table 1), and the stations sampled with the ORI plankton net (red circles; Stations O1–O17) to collect eggs and newly hatched preleptocephali (D) during the YK17-10 cruise of the R/V YOKOSUKA in May 2017.

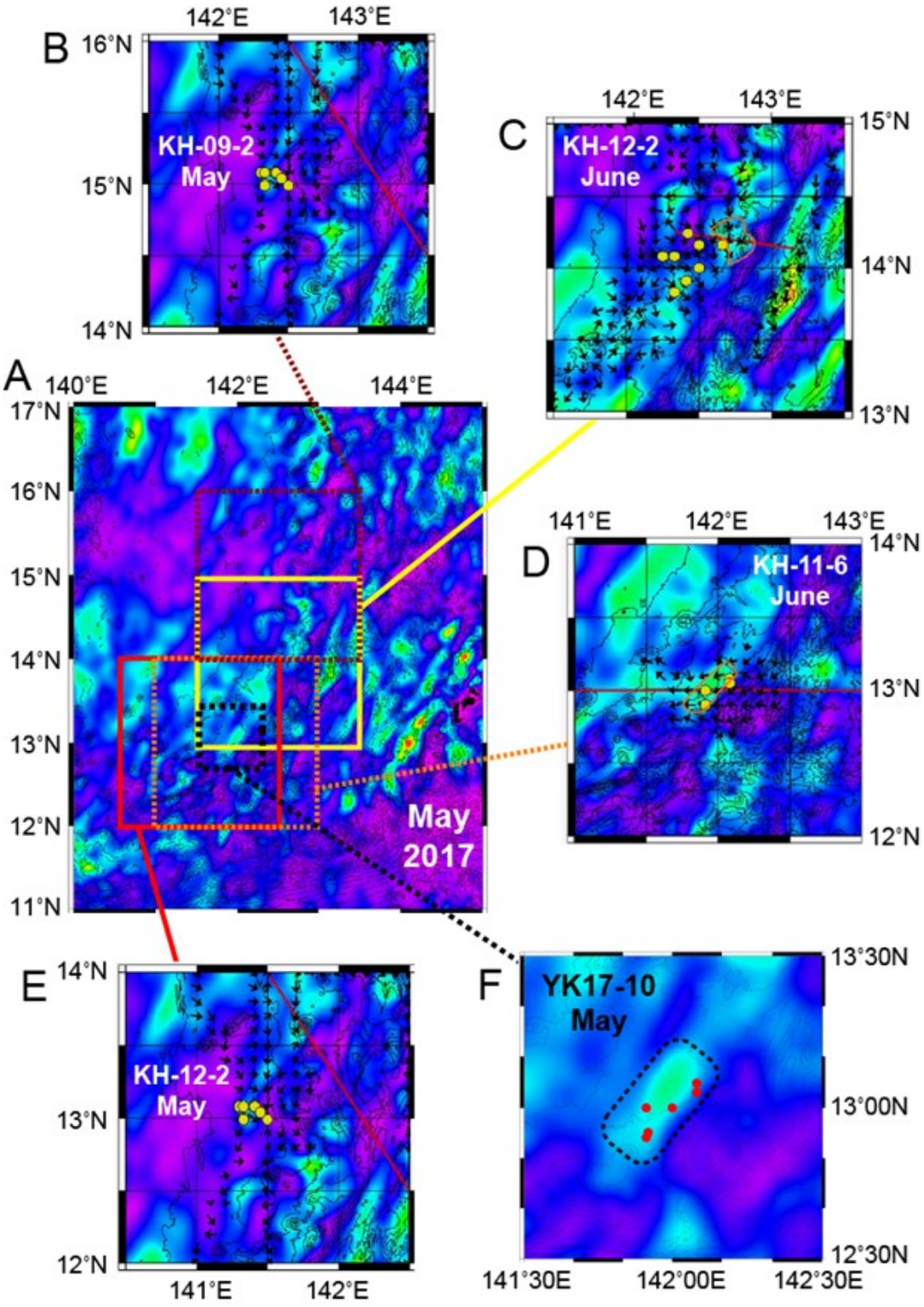

Figure 3. Map showing the patterns of model-simulated internal tide energy (Niwa and Hibiya 2014) during the YK17-10 cruise of the R/V YOKOSUKA in May 2017 (A, F) and internal tide patterns in relation to the locations of egg collections (yellow circles) during previous cruises. The patch of high internal tide energy (dashed oval) that was set as the observation area of the YK17-10 cruise (F) is shown along with egg collection locations in 2011 (D) and during NT14-09. Higher internal tide energy is shown by yellow and green color.

3. Observations

3.1 Purpose, Objectives, Background

The May 2017 cruise YK17-10 of the R/V YOKOSUKA was designed to improve the understanding of the spawning ecology of Japanese eels, *Anguilla japonica*, by taking the research efforts on this species to a new level of precision using several types of recent technological advancements. One important advancement is that totally new information about the depths at which anguillid eels may reside in their spawning area was obtained from eels tagged with satellite transmitting pop-off tags (PSAT) released during the NT14-09 cruise of the R/V Natsushima in 2014 (NT14-09 Cruise Report). PSAT tags have been used to learn about the migratory behavior of several species of anguillid eels recently, including Japanese eels earlier in their offshore migrations (Manabe et al. 2011), New Zealand longfin eels (Jellyman and Tsukamoto 2005, 2010), European eels (Aarestrup et al. 2009; Righton et al. 2016), American eels (Béguer-Pon et al. 2015), and tropical South Pacific eels (Schabetsberger et al. 2013, 2015). These studies all show that anguillid eels show distinct behaviors of diel vertical migration (DVM) in which they swim in the upper few hundred meters at night and then much deeper during the day (see Schabetsberger et al. 2016). In the case of the Japanese eels released in their spawning area in 2014, the data showed that the eels would likely be at depths where temperatures were about 4.7–5.2°C during the day at depths reaching between about 700–890 m, and at shallower depths of around 200–250 m during the day as shown in Figure 4 (Watanabe, Higuchi, Tsukamoto, unpublished data). Therefore, these teperatures/depths could be targeted for Shinkai 6500 observations during the day and by YKDT observations at night.

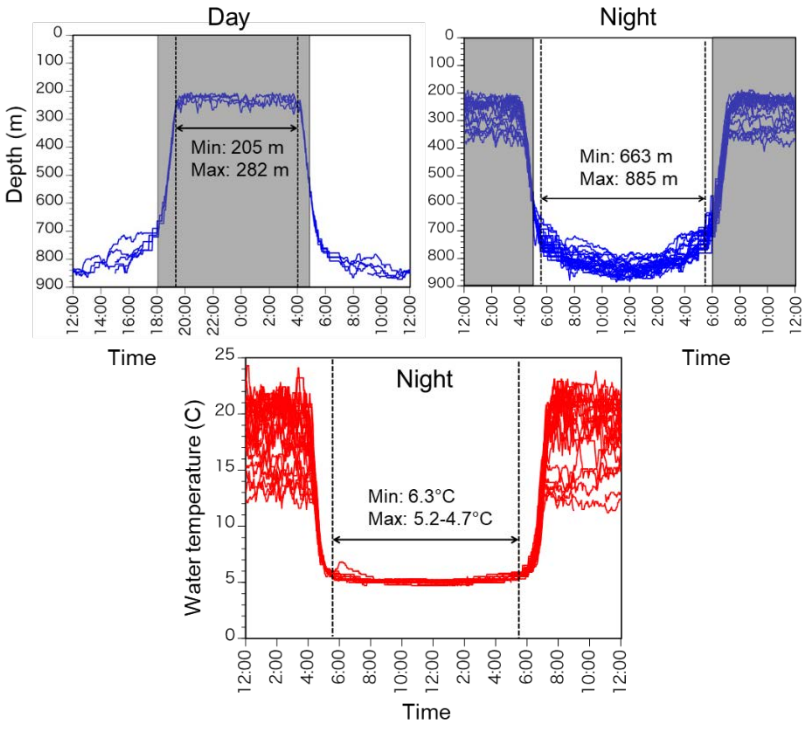

Figure 4. Swimming depths during day and night (top panels) and temperatures experienced at night (lower panel) of an eel tagged and released in the spawning area in 2014, that were used to guide the observation depths of the YK17-10 Shinkai 6500 (day) and YK-DT (night)

In addition, the cutting-edge research approach of analyzing environmental DNA (eDNA) from water samples, can now be used to detect the presence of Japanese eels at various depths, after their DNA was detected during the NT15-08 cruise (NT15-08 Cruise Report). This technique can be used to evaluate the presence of Japanese eels in both horizontal and vertical axes by analyzing eDNA at different station locations and at different depths using quantitative onboard Real-Time PCR (RT-PCR). eDNA is being increasingly used to evaluate the presence or abundance of organisms in both freshwater and marine environments (Minamoto et al. 2012; Takahara et al. 2012; 2013; Thomsen et al. 2012, 2016; Kelly et al. 2014; Yamamoto et al. 2016).

The third new technique used during the present survey was the distribution of internal tidal energy using a model created by University of Tokyo scientists (Niwa and Hibiya 2014). Previous catches of Japanese eel eggs along the West Mariana Ridge (Aoyama et al. 2014), were each found to have occurred near high-energy patches of internal tides (Figure 3) and have been hypothesized to be detected by eels and then used as landmarks of aggregation areas (Higuchi et al. 2015). This might serve to help eels know where to find each other, but may also result in increased local primary productivity and therefore increase the food available to their newly hatched larvae in these areas.

These new techniques were combined with previous information about the spawning timing and locations that were used to guide previous attempts to observe eels in the spawning area. These efforts led to the short sighting of a possible anguillid eel during YK12-11 (Tsukamoto et al. 2013), observations of mesopelagic eel reproduction-related behavior by the drifting Una-Cam observation systems during NT13-11 (Fukuba et al. 2015; NT13-11 Cruise Report), observations of other marine animals (Tsukamoto et al. 2013; Miller et al. 2014, 2015), and collections of eggs and preleptocephali in the survey area during NT14-09 (NT14-09 Cruise Report). Those surveys were guided by the fact that collections of newly spawned preleptocephali (Tsukamoto 2006; Tsukamoto et al. 2011; Kurogi et al. 2011; Aoyama et al. 2014), eggs (Tsukamoto et al. 2011; Aoyama et al. 2014), and spawning-condition male and female eels along the southern part of the West Mariana Ridge (Chow et al. 2009; Tsukamoto et al. 2011; Kurogi et al. 2011) all indicate that the Japanese eel only spawns during the fuew days just before new moon. This hypothesis was originally formulated from otolith daily ring analyses of various sized of leptocephali, which all had back-calculated hatching dates near new moon periods during the spawning season (Ishikawa et al. 2001; Tsukamoto et al. 2003). The other factor used to guide this and previous surveys is that the position of a shallow salinity front within the spawning area appears to affect the latitude of spawning of the Japanese eel (Tsukamoto 1992; Kimura and Tsukamoto 2006; Tsukamoto et al. 2011). It appears that when a distinct front forms, spawning occurs just south of the front, but when there is no distinct front and

low salinity extends across the region, eels can spawn at a range of latitudes (Aoyama et al. 2014).

Therefore, the YK17-10 cruise was designed to use the location of the salinity front and internal tide patches to determine the survey area in the days before new moon, and to use the PSAT swimming depths and any e-DNA positive detections to guide the observation depths of the 2 types of underwater camera systems (Shinkai 6500 during the day and YKDT at night). Water samples were also to be taken for later amino acid analyses for the ongoing study of the chemical environment of the spawning locations of the Japanese eel (Okino et al. unpublished data). Then at the end of the cruise the ORI plankton net was used to determine where spawning was occurring by attempting to catch any remaining eggs or the newly hatched preleptocephali. The NT15-08 survey was also designed to use a similar set of factors to guide the sampling, but two typhoons reduced the sampling time to 2 days (NT15-08 Cruise Report), so the present cruise is the first test of the new set of factors that can guide the effort to observe spawning eels for the first time and to increase the understanding of what determines where they decide to spawn. Fortunately, as described below, the weather was not a factor in 2017, and all of the planned observations were carried out which led to the first observation of an anguillid eel in its spawning area anywhere in the world, multiple detections of Japanese eel e-DNA for the first time, and various other important and interesting observations of marine eels and a rare deep-sea octopus that will lead to multiple publications.

3.2 Observations and Activities

The research effort during the YK17-10 cruise included several types of observation methods that were conducted in a sequential or overlapping order: (1) Examination of the salinity structure of along the West Mariana Ridge using X-CTD probes for plotting salinity sections to determine the observation area, (2) Deciding an area of likely spawning based on the salinity front and tidal energy patterns, (3) Deployment of the Shinkai 6500 during the day and the YKDT at night to attempt to film spawning eels, (4) e-DNA analyses of water samples from the Niskin bottles that were newly attached to both the Shinkai 6500 and YKDT to provide additional information about where to make observations for eels, (5) Simultaneous collection of samples for later amino acid analyses, (6) Sampling with the ORI plankton net to attempt to collect recently spawned Japanese eel eggs or more likely preleptocephali to evaluate where spawning had occurred.

3.3 Methods and Instruments

The expendable conductivity, temperature and depth profiler probe (X-CTD; Tsurumi-Seiki Co., LTD) survey began at the northern end (Stn. X23) of the transect of

hydrographic stations that extended along the eastern side of the West Mariana Ridge and continued until the southernmost station was reached (Stn. X12) (Figure 1). Then X-CTD deployments continued while the ship was moving north along the western line until Stn. X7. No further stations were sampled because both the eastern and partial western hydrographic sections were sufficient to understand the salinity structure. Each X-CTD deployment recorded data to a depth of about 1000 m.

The hydrographic sections indicated there was a deeper pool of low salinity water in the south that formed a partial salinity front (Figure 5). This pool of water was clearly seen in horizontal sections plotted from the X-CTD data (Figure 6). Analyses of the internal tide patterns using the model of Niwa and Hibiya (2014) showed that there was a strong patch of internal tide (Figure 2,3A,F) within that low salinity pool, so the observation area was set to cover the patch. This location was very near where Japanese eel eggs were collected in June 2011 (Figure 3D,F; Aoyama et al. 2014) and where the first anguillid eel adults were collected (the "Kaiyo Point"; Chow et al. 2009; Tsukamoto et al. 2011), so it seemed likely that spawning could occur there in 2017.

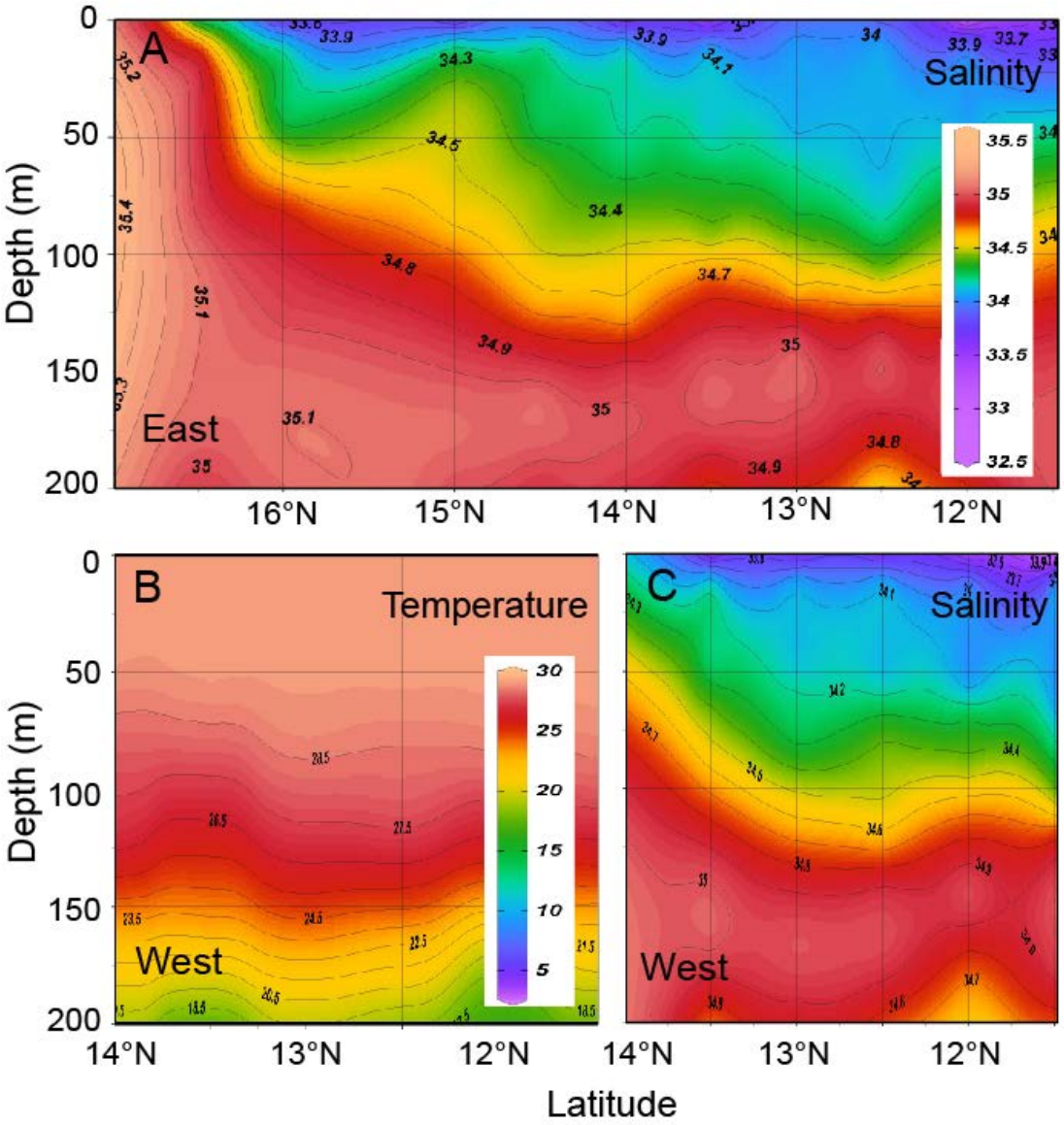

Figure 5. Hydrographic sections of salinity along the eastern side of the seamount ridge (A) and temperature and salinity along the west side of the southern part of the ridge (B,C) before the intensive observation period of the YK17-10 cruise.

A plan was made for Shinkai 6500 and YKDT deployments to occur from 4 directions across the internal tide patch to search for eels, and to collect water samples for e-DNA analysis during each deployment of both types of observation systems (Figure 2A,B). Video imagery was recorded from a variety of cameras on both underwater vehicle systems (Figure 7), and scientists and staff members watched the live video feeds and made notes about any organisms that were observed (Figure 8). The submersible had 2 video cameras and a camera for photographs, and the YKDT had 3 video cameras, and one camera to take photographs. The YKDT was also deployed during the day at a grid of stations covering the internal tide patch (Figure 2C) for water sampling, to record CTD profiles, and to record video that could be observed after the cruise. For the underwater video observations, the plan was to deploy the Shinkai 6500 at temperatures during the day according to where eels may be based on the PSAT tagging data that showed a preference for the temperature range of about 4.7–5.2°C at depths of about 700–880 m (Figure 4) (915–971 m in the X-CTD data from Stn. X9). The YKDT was deployed at depths of 200–250 m according to where eels may be at night (Figure 4). The results of the e-DNA analyses could then also be used to guide the observations.

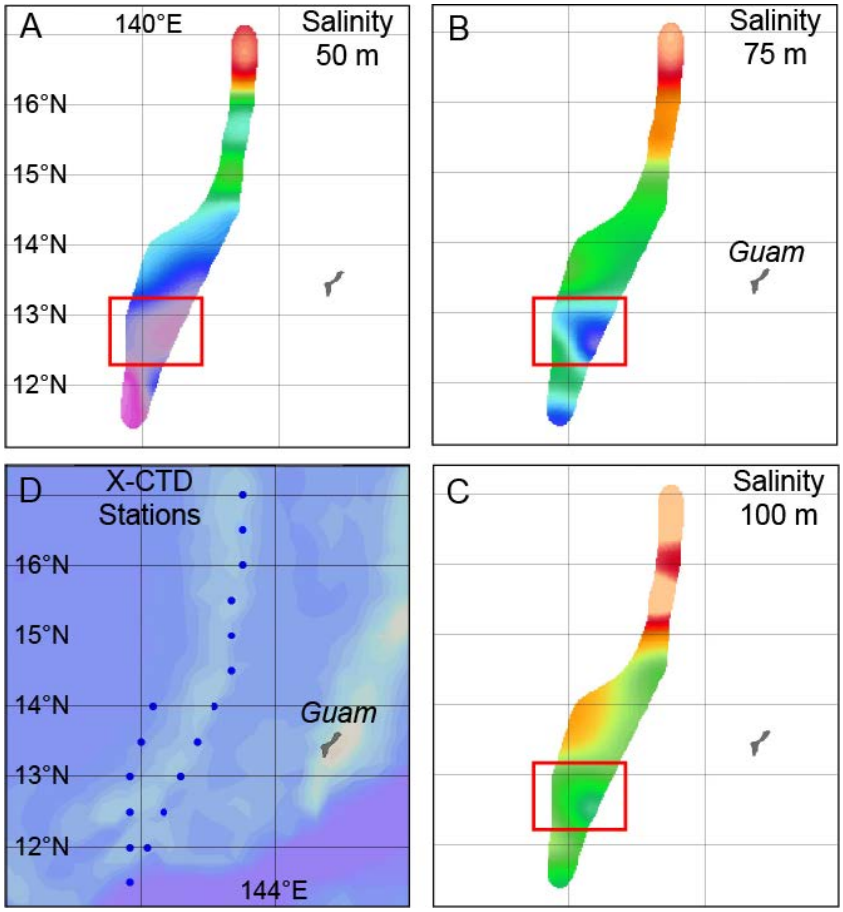

Figure 6. Horizontal sections of salinity plotted from the X-CTD data showing the deep pool of low-salinity water that was present in the area (red box) where the observations of the YK17-10 cruise were made in May 2017.

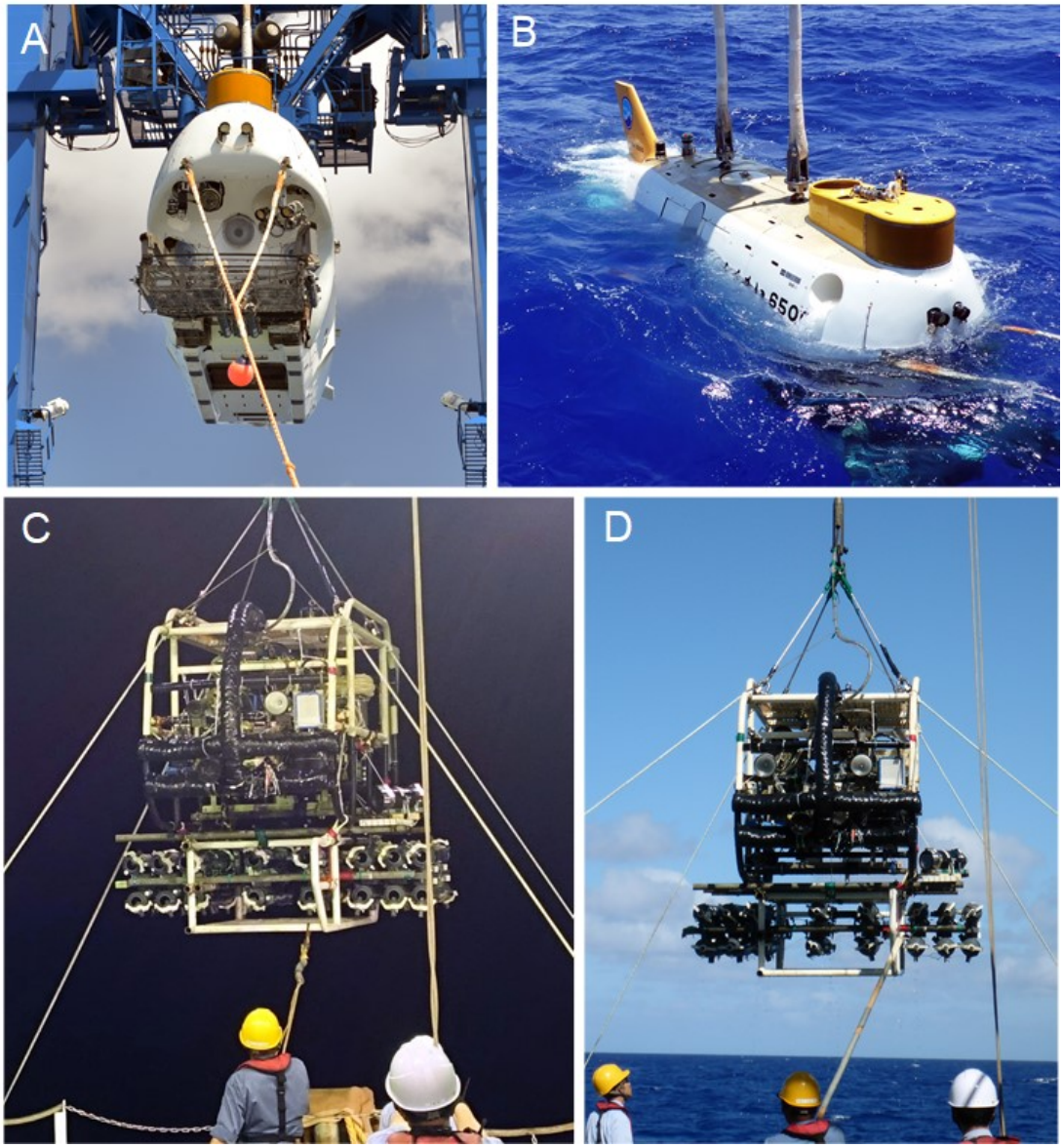

Figure 7. The Shinkai 6500 submersible (A, B) and the YKDT towed camera system (C,D) being deployed during the YK17-10 cruise in May 2017. Both underwater observation systems had multiple camera systems, and Niskin water collection bottles were attached to collect water samples.

The seawater samples for eDNA and amino acid analyses were taken from 8 CTD-type Niskin bottles attached in the front region of the Shinkai 6500 (1 on each side and 6 in the middle; Figure 9) and from 16 bottles attached below the YKDT (Figure 7C,D). Water samples were taken from 50, 100, 150, 200, 400, 600, 800, and 1000 m depths for eDNA analyses to estimate the presence of Japanese eels. The water from the

bottles was transferred to 10 liter bottles for eDNA filtration and smaller bottles for amino acid analyses immediately after the two systems came back onboard (Figure 9).

Figure 8. Photographs of the scientific observation areas where the video imagery transmitted from the Shinkai 6500 submersible was observed onboard the ship during each daytime dive and events recorded as they occurred (A-C), and the multiple screens of direct video feeds through the real-time cable of the YKDT that were watched by scientists and YKDT staff at night (D, E) during the YK17-10 cruise in May 2017.

The water samples for eDNA analyses were filtered (Figure 10A) through Sterivex units with a pore size of 0.45 µm (Toyo, Japan, Merck Millipore) following the methods of Miya et al. (2016). In addition to the CTD seawater samples, 10 L samples of Milli-Q water were filtered to analyze as negative controls. Filters were stored in a freezer (-20ºC) until DNA extraction. DNA extraction and quantitative real-Time PCR onboard the cruise was conducted to attempt to detect eDNA from Japanese eels (Figure 10B). Total DNA on the filters was extracted using DNeasy blood & tissue kits (Qiagen). The total DNA was then subjected to quantitative real-Time PCR onboard using a LightCycler® Nano (Roche) Real-Time PCR analyzer in triplicate. The protocol was to target the 16s ribosomal RNA gene of the mitochondrial genome (mtDNA) according to previously established protocols (Watanabe et al. 2004; Minegishi et al. 2009). The thermal profile of the Real-Time PCR was 95ºC for 600 s, and 50 cycles of 95ºC for 20 s, and 60ºC for 40 s. The remaining filters collected from the Shinkai 6500

water samples for eDNA analyses were transported frozen to Nihon University in the laboratory. Samples for amino acid analyses were also processed onboard during the cruise (Figure 10C), which will be analyzed later at Hokkaido University as described previously (NT14-09Cruise Report).

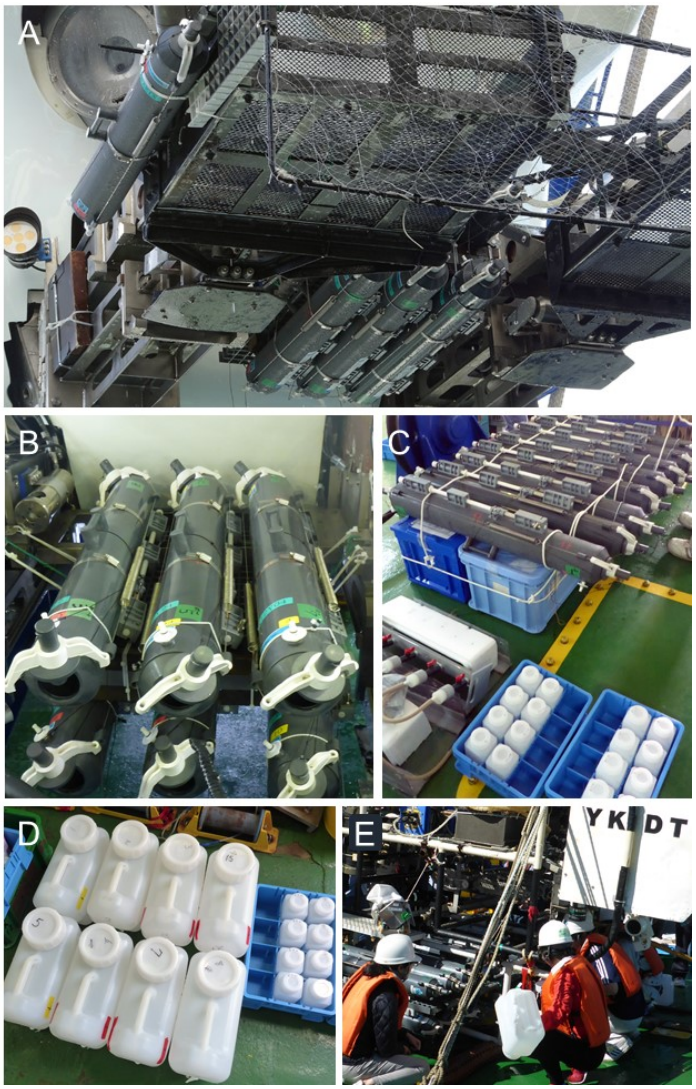

Figure 9. Locations of the Niskin bottles on the front and side of the Shinkai 6500 submersible (A, b), the bottles arranged in preparation for collecting water samples for filtration (C), the large bottles used to collect water samples for eDNA filtration and the small bottles for amino acid analyses (D), and water samples being collected from the YKDT Niskin bottles.

After the underwater observations and water sampling was finished, the ORI plankton net was used to survey for late-stage eggs or newly hatched preleptocephali (Figure 11). The ORI plankton net was 1.6 m in diameter with 0.33 mm mesh and was towed horizontally at the 4 depth-layers of 200–190 m, 160–150 m, 120–110 m, and 80–70 m. The net was released down to a depth of 200 m with a ship speed of 2.5 knots and a wire speed of 1.0 m/sec, then it was towed horizontally at each of the

depth-layers for 10 min. The net was deployed at 18 stations. The plankton samples were then sorted fresh onboard to find any eggs or preleptocephali, with the remainder of the samples being preserved in 10% formalin-seawater. The eggs, preleptocephali and leptocephali that were collected were preserved in 99% ethanol after identification.

Figure 10. Photographs of water filtering for eDNA analysis (A), DNA extraction for RT-PCR (B), preparation of amino acid water samples for later analysis (C), and the RT-PCR analysis apparatus (D) during the YK17-10 cruise.

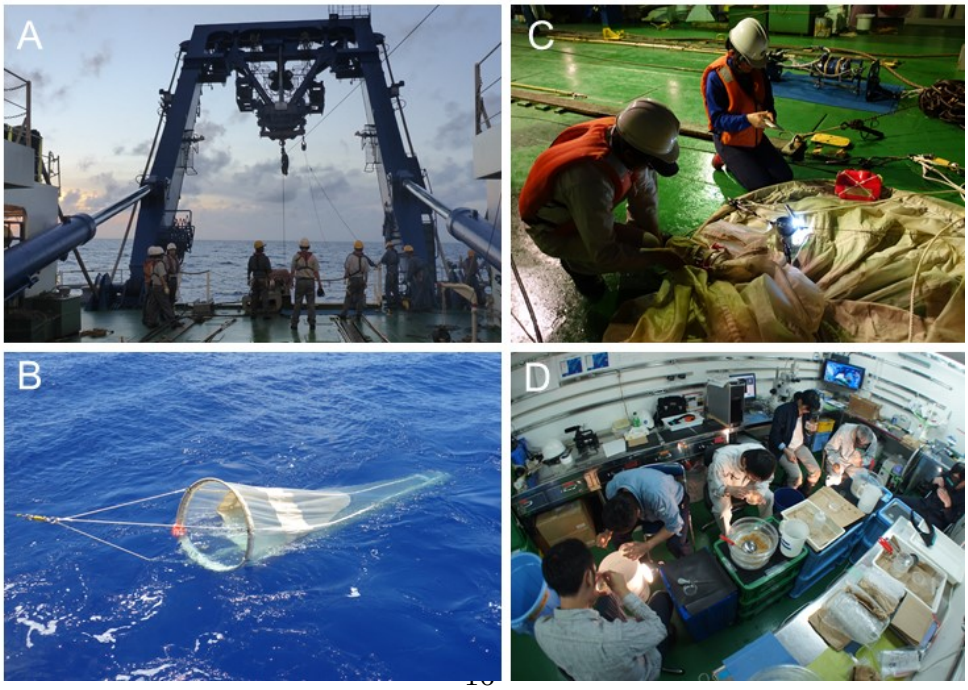

Figure 11. Photographs taken during the ORI plankton net sampling (A-C), and sorting of the plankton samples (D) during the YK17-10 cruise in May 2017.

4) Research results

4.1 Hydrographic structure and internal tide patterns

The salinity section plotted from the transect of X-CTD stations along the eastern side of the West Mariana Ridge indicated that a shallow layer of low-salinity water (<34.5; Aoyama et al. 2014) extended to the north past 16°N, but it did not extend as far north on the western side of the ridge (Figure 5B). On the west side, a front formed to the north of 13°N and the deepest pool of low-salinity water near the front was present just south of 13°N as seen in the horizontal salinity sections (Figure 6). This area also included a patch of high internal tide energy as seen in Figure 3F. There was a uniform mixed layer of 28°C water in the upper 75 m that extended past 14°N (Figure 5), but the mixed layer became shallower to the north and disappeared at about 16°N where the low-salinity water ended (not shown). The subsurface layer of high-salinity Subtropical Underwater (see Schabetsberger et al. 2016) was present across the area with its core being at a depth of about 170 m at the 13°N observation area. Therefore, the combination of a strong internal tide patch being present south of a salinity front was used to determine the location of the intensive underwater observations and eDNA analyses that were centered over the internal tide patch (Figure 2).

4.2 Shinkai 6500 deployments

There were 5 dives made by the Shinkai 6500 submersible during 19–24 May to depths between 400–1000 m within the study area that was centered over the internal tide patch (Figure 2). Each dive had a different pattern of survey methods for the horizontal tracks they made (Figure 12) and the depths that were occupied (Figure 13). The first and second dives (#1492, 1493) surveyed in one linear direction mostly following the depth of the 4.7–5.2°C temperature range that was determined from the PSAT data of a tagged Japanese eel as described previously (Figure 4). Therefore, most of the diving time was spent between 800 and 1000 m. No dive was made on the day after the first dive to allow for intensive eDNA water sampling using the YKDT. The third dive (#1494) went down to 900 m and then moved around a central area in concentric squares while moving up to make observations at 3 shallower depths before going back down to 800 m near the end of the dive. Dive #1495 went down to 1000 m and then moved steadily up in short increments of depths to survey at many different depths along a linear track. The final dive (#1496) first surveyed at 400 m where a strong eDNA signal was detected the day before, then went down to 1000 m, up to 400 m and down to almost 800 m during a linear track dive with one right angle turn.

During the descents of the submersible down to 1000 m, a light meter was used to measure the changes in light intensity. There is little data about the relationship between daytime light levels and the ecology of eels, so this was a first a first step to gather direct data on light levels in relation to depths where all life history stages are present.

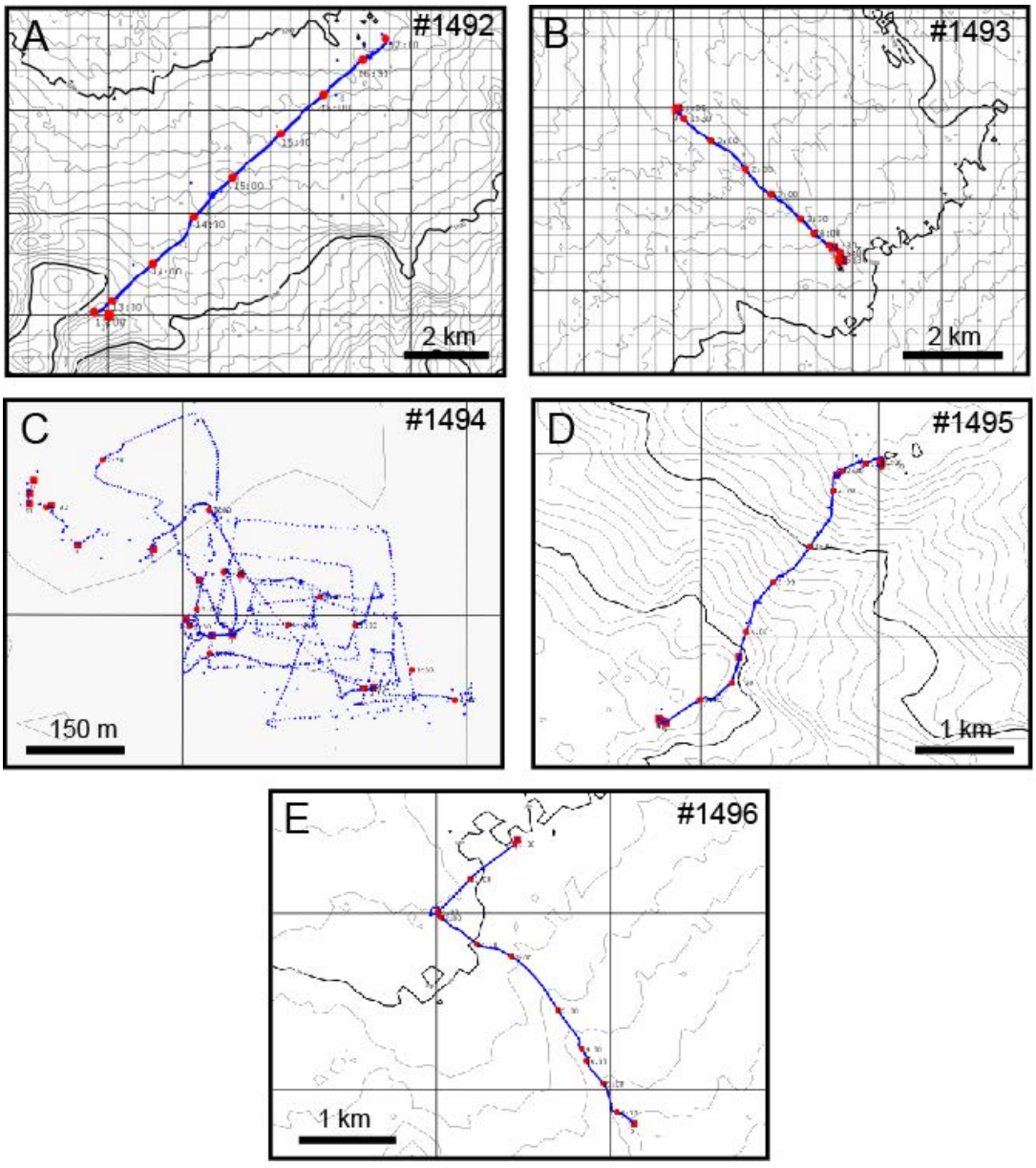

Figure 12. Horizontal plots of the dive tracks of the Shinkai 6500 submersible during the 5 dives made during the YK17-10 survey in May 2017 (also see Figure 2A).

During these dives plankton and marine snow were continuously seen in the video recordings and a variety of larger animals were occasionally seen, although no Japanese eels were seen during the Shinkai 6500 dives. The most distinctive fishes to be seen were mesopelagic eels of the family Serrivomeridae, which were holding position with

their bodies in a rigid vertical position. The first vertical eel was seen during dive #1492 at a depth of about 850 m, and another eel was seen about 40 min after that at almost the same depth of 849 m (Figure 13). Those eels were in a head-down or head-up orientations and only swam away when the submersible was very close to them (Figure 14A,B,D). Another vertical eel was seen at 706 m during dive #1495 that was in a head-up position and had an enlarged body cavity, suggesting it had recently eaten food, or may have been gravid with eggs (Figure 14C). Three other eels with head-up orientations were seen by the scientist observer during that dive at about 650, 750, and 800 m depth ranges, and some were also seen during dive #1493. Fish, shrimp and gelatinous zooplankton were sometimes seen when these eels were seen, so those depths may be good depths for feeding during the daytime. During the previous eel survey using the Shinkai 6500 in 2012 only one dive was made to these deeper depth ranges (Tsukamoto et al. 2012), but a vertical eel was also seen during that dive (Miller et al. 2014). Eels using that vertical body position may be common along the West Mariana Ridge, which will form the basis of a new paper about the behavior of these eels.

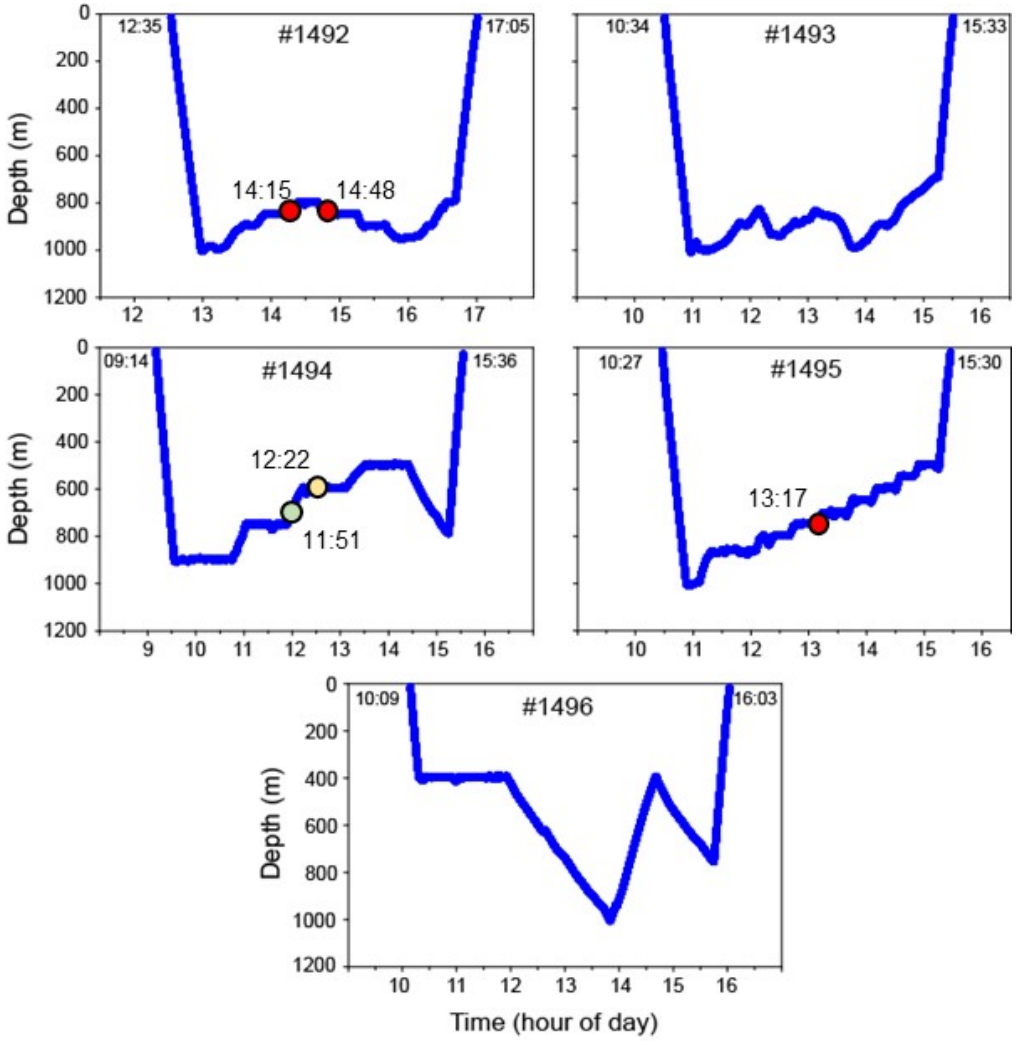

Figure 13. Plots of the depths occupied by the Shinkai 6500 submersible during the 5

dives made during the YK17-10 survey showing the locations and times when the vertically oriented serrivomerid eels shown in Figure 14 were seen (red circles), and when the *Nessorhamphus* eel (green circle; Figure 15A) and the seven-arm octopus (yellow circle; Figure 15C) were seen.

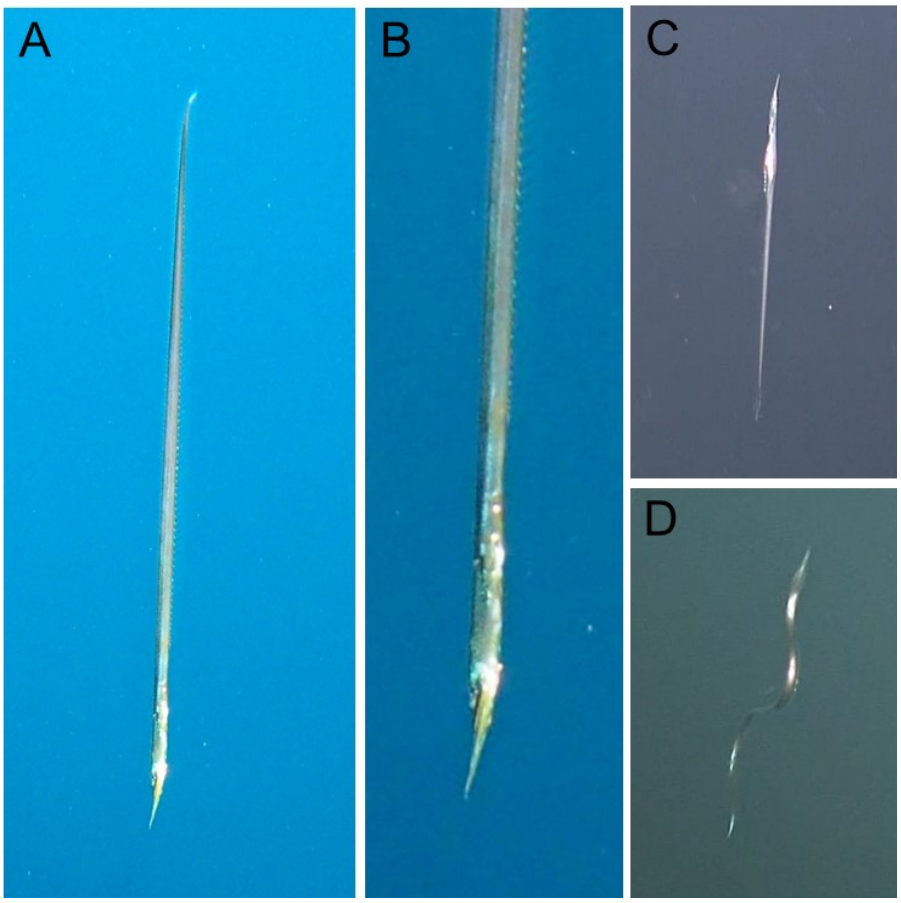

Figure 14. Images of mesopelagic eels of the family Serrivomeridae that were holding position with their bodies either in a rigid vertical position with a head-down orientation (A, B; dive #1492 at 850 m) or a head-up orientation (C; dive #1495 at 706 m), and of an eel that was beginning to swim away after using a head-up orientation (D; dive #1492 at 849 m).

Another distinctive species observed by the Shinkai 6500 during dive #1494 was a large deep-sea octopus that was video recorded as it stayed in front of the submersible (Figure 15C) at a depth of about 590 m (Figure 13). The octopus may have initially moved away as the submersible approached it, but it then remained mostly motionless for a while in front of the submersible enabling high-quality video recordings to be made. Based on those imagery, the octopus quite clearly seems to be a rare pelagic seven-arm octopus, *Haliphron atlanticus* Steenstrup, 1861, also previously named *Alloposus mollis* Verrill, 1880.

Haliphron atlanticus is a circumglobal species that lives in the open ocean and has

also been reported near continental slopes at shallower depths, but does not seem to have been reported from the western North Pacific (Lima et al. 2017). This semi-gelatinous octopus species appears to feed on gelatinous zooplankton (Hoving and Haddock 2017). It is preyed upon by predators such as sperm whales and blue sharks, so some reports of its presence are of body fragments likely resulting from predation.

Existing observations of *Haliphron atlanticus* include a large female that was previously video recorded near the bottom with its egg clusters at 270 m at Hawaii (Young 1995), and another individual was seen more recently at 396 m at Hawaii (https://www.youtube.com/watch?v=sw8zl5vrAu8&feature=youtu.be&%20t=1m50s). Smaller individuals were also seen by ROV cameras at Hawaii and in Monterey Bay including one holding a jellyfish in its mouth at 378 m (Hoving and Haddock 2017). https://www.youtube.com/watch?v=CzU8CUXxLsA&feature=youtu.be). The different habitat of the deep-sea, the lack of reports from the region, and the high quality of the video obtained of this species during YK17-10 indicate that this unique observation should be published.

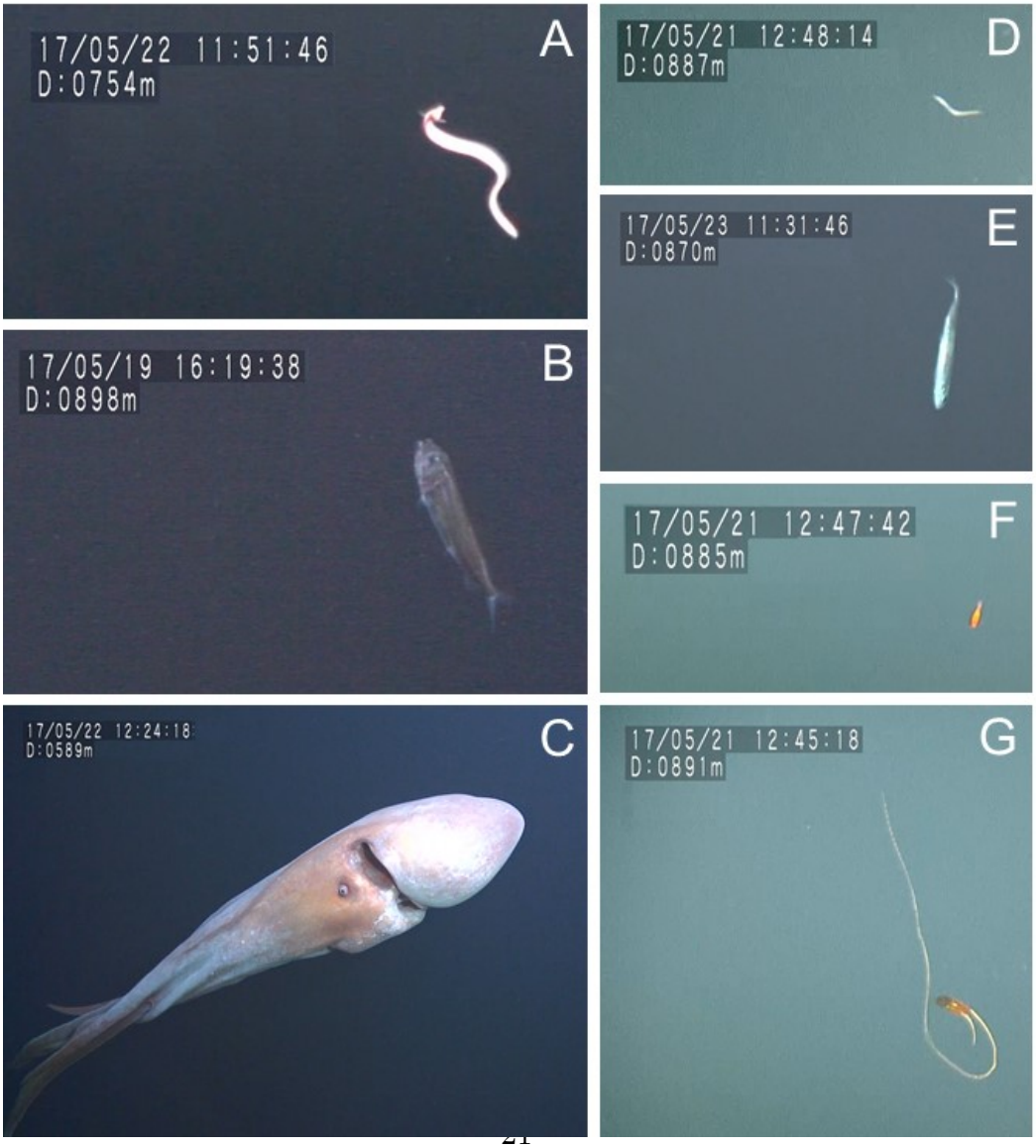

Figure 15. Various marine animals observed by the Shinkai 6500 submersible during the YK17-10 survey, showing a mesopelagic duckbill eel of the genus *Nessorhamphus* (Derichthyidae) seen at 754 m (A), small fishes (B,E), a rare pelagic seven-arm octopus, *Haliphron atlanticus*, at 589 m (C), and an unidentified organism (G).

Other smaller animals were also seen, such as a mesopelagic derichthyid duckbill eel of the genus *Nessorhamphus* seen at 754 m (Figure 15A), a small fish with a vertical orientation at 870 m (Figure 15E), and a deep-sea fish at 885 m (Figure 15B). Jellyfish, siphonophores, and shrimp were seen, as well as an unidentified apparent invertebrate of some kind at 891 m (Figure 15G). The video recording of the duckbill eel of the genus *Nessorhamphus* may be the first of its kind.

4.3 YKDT deployments

The YKDT video cameras also recorded various marine animals at night, including gelatinous zooplankton, small fishes, and squid, and it was eventually discovered that an anguillid eel had been recorded (Figure 16). Fewer species and individuals of fishes and squid seem to have been clearly observed possibly because there was no downward looking camera as a result of the Niskin bottle array for water sampling being attached below the YKDT. It was the downward looking camera that enabled many fish and squid to be seen in the 2012 deployments (Tsukamoto et al. 2012), so various individuals may have been residing in the lighted area below the YKDT, but could not be observed during YK17-10.

Figure 16. Frame captures of an anguillid eel recorded at 21:42 during the YKDT dive #192 on 20 May during the YK17-10 survey while the YKDT was at a depth of 222 m (Figure 17,18).

No anguillid eels were clearly seen while watching the real-time video streams on the TV monitors (Figure 8) onboard during the nighttime deployments, although notes were taken about anything that was observed, even if it was not clear what was seen.

There was limited time to review all notes and check the video files while the survey was ongoing. However, one note was checked on the last day of the cruise after observations ended, which showed that at 21:42 on 20 May during the YKDT dive #192, a large slender anguillid eel was observed to be swimming in the opposite direction that the YKDT moving, just below and to the left of the YKDT, which was at a depth of 222 m (Figure 16). It was recorded in both the YKDT HD camera and a separate camera attached to the side of the YKDT for use by the NHK team. The eel was elongate with a large tail visible near the end of the video recording. That tail shape is a key factor to distinguish between anguillid eels and those of the mesopelagic eel family Derichthyidae (Tsukamoto et al. 2012). The body shape and swimming style of the eel also appeared to be that of an anguillid eel and not a derichthyid eel or any other type of more elongate mesopelagic eel that have different body forms.

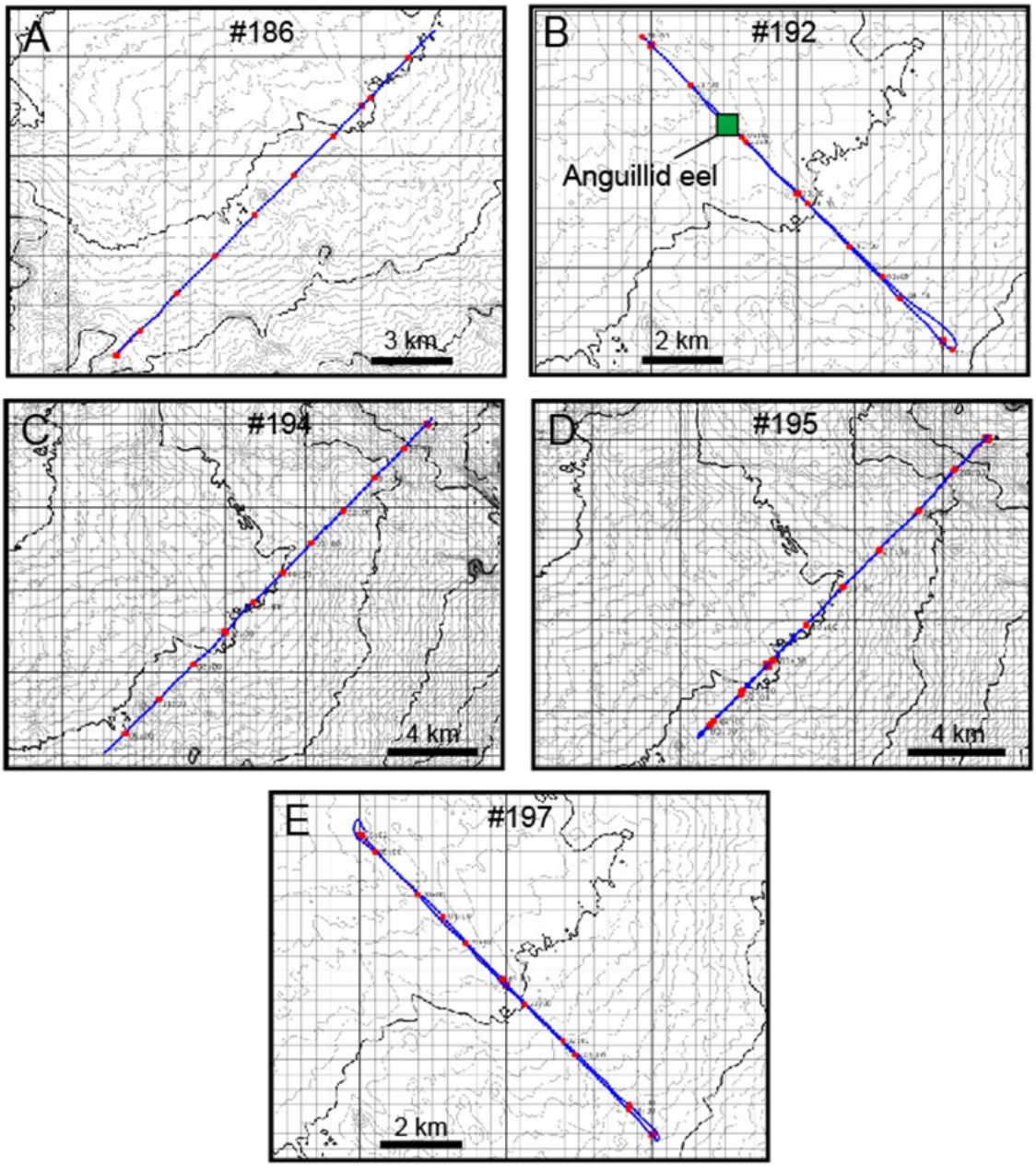

Figure 17. Dive tracks of the 5 nighttime YKDT observation dives showing the location where an anguillid eel was seen (green square) while the YKDT was at a depth of 222 m during the YK17-10 survey in May 2017 (Also see Figure 2B).

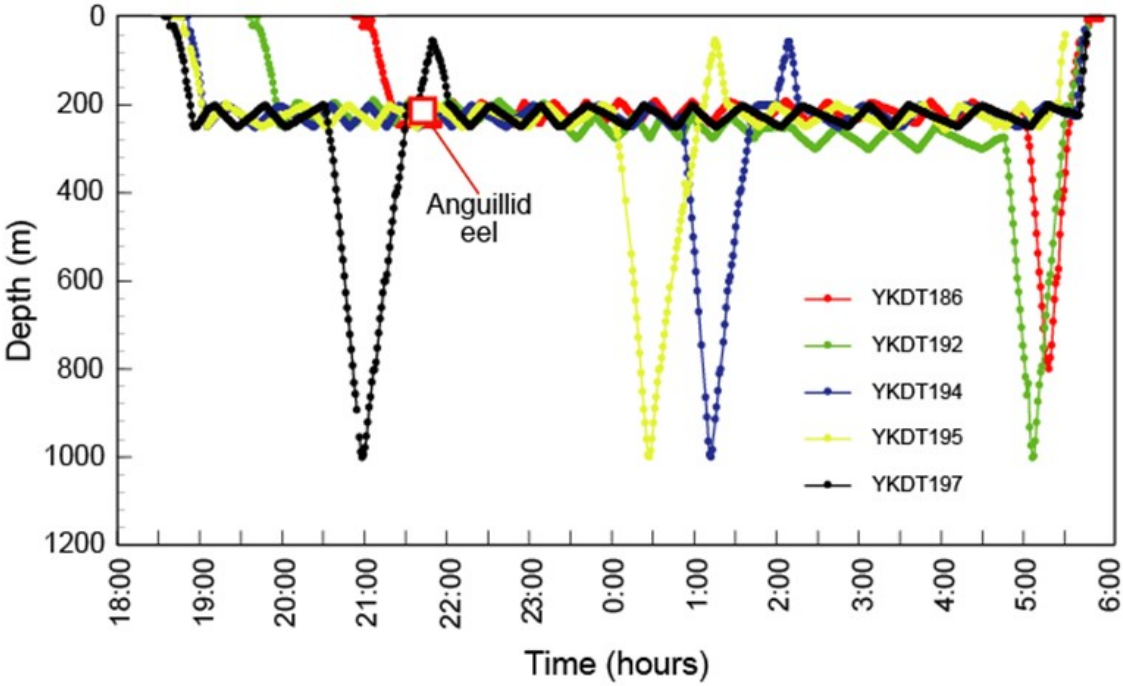

Figure 18. Plots of the depths surveyed by the YKDT observation system during the 5 dives made at night to observe eels. The time an anguillid eel shown in Figure 16 was recorded at 21:42 during dive #192 is shown with the white square. Deep depths were reached during each dive to record CTD data and collect water samples.

The eel had whitish colorations that may indicate it was in poor condition after being in spawning condition for a while, which is consistent with the appearance of some of the anguillid eels collected in trawls in this area (Chow et al. 2009; Kurogi et al. 2011; Tsukamoto et al. 2011). The recording was not high enough resolution to clearly distinguish which anguillid species it was, but it did not have an enlarged abdomen, so was not a pre-spawning female and may have been a male eel. There does not appear to be clear evidence to indicate if was a Japanese eel (*Anguilla japonica*) or small giant mottled eel (*Anguilla marmorata*), whose adults have also been collected by trawling in this area (Tsukamoto et al. 2011) and that spawn in an overlapping area with Japanese eels (Kuroki et al. 2009).

This video recording that includes the presence an anguillid eel represents the first known underwater observation of an anguillid eel in its spawning area anywhere in the world. It also may provide the first direct evidence about the depths at which anguillid eels search for other eels to form spawning aggregations.

4.4 Water sampling and environmental DNA analyses

Seven YKDT deployments were made primarily for collecting CTD data and seawater samples for eDNA and amino acid analyses (dives #187, #188, #189, #190, #191, #193, #196) that resulted in 63 samples being collected for filtering eDNA (56 water samples and 7 negative controls). In addition, 44 samples were obtained (39 water samples and 5 negative controls) from 5 dives of the YKDT made for nighttime video observations (dives #186, #192, #194, #195 and #197). Seawater samples were primarily collected at 8 water depths of 50, 100, 150, 200, 400, 600, 800 and 1000 m. There were also 32 water samples (4 negative controls) collected from 4 of the Shinkai 6500 dives (dives #1492–1495) at the same 8 water depths.

Table 1. Results of the Real-Time PCR of the filtered water samples collected by the YKDT during the YK17-10 cruise showing the depths of the samples, station numbers, days before new moon (nm) and other details. Positive results of the PCR (crosses indicating replicates, yellow and red colors) were obtained at 6 stations and at 4 depths.

Date of May	20	20	20	20	20	20	21	21	22	23	23	24
Days before	-6	-6	-6	-6	-6	-6	-5	-5	-4	-3	-3	-2
Time	5:27	8:47	11:08	13:33	15:45	17:49	5:23	7:37	1:32	0:53	7:54	21:22
YKDT dive	#186	#187	#188	#189	#190	#191	#192	#193	#194	#195	#196	#197
Station	St. 3	St. 4	St. 5	St. 2	St. 1	St. 1	St. 3	St. 3	St. 3	St. 3	St. 3	St. 3
50 m	-	-	-	-	-	-	-	-	-	-	-	-
100 m	-	-	-	-	-	-	-	-	-	-	-	-
150 m	-	-	-	-	-	-	-	-	-	-	-	-
200 m	-	-	-	-	-	-	-	-	-	-	-	+
400 m	-	-	-	+	-	-	-	+*	-	-	+++	-
600 m	-	+	-	-	-	-	-	-	-	-	-	-
800 m	-	-	+	-	-	-	-	-	-	-	-	-
1000 m	NS	-	-	-	-	-	-	-	-	-	-	-

NS: no sampling; *negative control showed eDNA amplification.

After the water samples were filtered, many were analyzed for the presence of Japanese eel eDNA. A total of 12 quantitative real-time PCR assays were conducted onboard during the cruise using 95 samples from 5 stations sampled by the YKDT and 12 negative controls. The remaining filters obtained from the Shinkai 6500 water samples were transported to Nihon University for eDNA analyses. Out of the 107 samples analyzed, 6 samples showed positive detections of Japanese eel eDNA. Positive samples were obtained from 200 m (1), 400 m (3), 600 m (1), and 800 m (1) at stations 2–5 (Figure 2C), with 3 of the positive detections being at Stn. 3 (Table 1). Each sample was tested in triplicate, and all except one of the positive samples showed

amplification of only one of the three replicates indicating low concentrations of eDNA. One sample from 400 m at Stn. 3 in the center of the internal tide patch however, showed amplification of all 3 replicates, indicating a high concentration of Japanese eel eDNA (Table 1).

To validate if these positive results were caused by the presence of Japanese eel DNA in the filters, the amplified PCR products will be further analyzed at Nihon University along with the remaining filter samples. The initial results however, suggest that Japanese eel eDNA was successfully detected during the YK17-10 cruise at depths where eels likely reside. This is also consistent with the video recording of an anguillid eel in the study area.

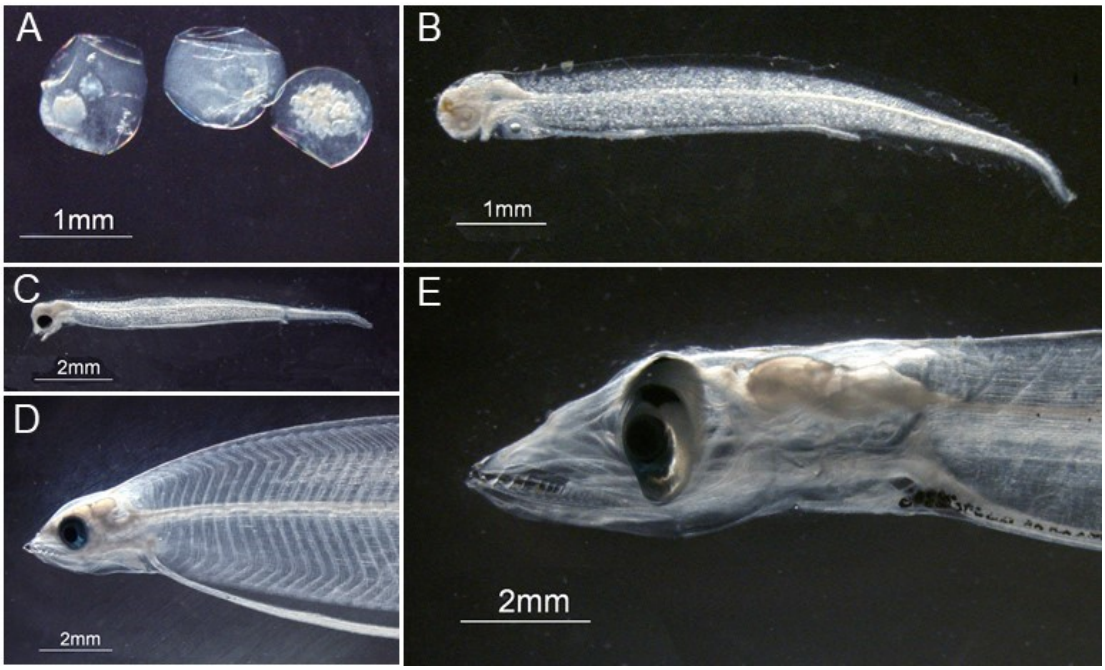

Figure 19. Photographs of eggs (A; Stn. O-2), preleptocephali with no eye pigment or teeth and an oil globule (B; 7.2 mm, Stn. O-8) or eye pigment and early teeth (C; 7.7 mm, Stn. O-14), the anterior body region of a 36.6 mm *Anguilla bicolor pacifica* leptocephalus collected at Stn. O-14 (D), and a 348.5 mm notacanthid (Albuliformes) leptocephalus collected at Stn. O-15 (E).

4.5 Net sampling for preleptocephali

After the underwater observations and water sample collection ended with the finishing of the 5th Shinkai 6500 dive, the research effort switched to fishing the ORI plankton net to collect any eggs remaining and the newly hatched preleptocephali. The net was deployed at 18 stations within a grid centered over the internal tide patch where underwater observations and water sampling was conducted. Then stations were sampled to the southwest where water from the patch may have been transported (Figure 2D). The ORI net deployments collected a total of 36 eel larvae and a variety

of fish eggs. One type of eggs that were usually damaged were collected at Stn. O-3 and other stations, which had egg shells with similar color hues of reflected light (Figure 19A) as Japanese eel eggs collected by the ORI net during the NT14-09 cruise (NT14-09 Cruise Report). However, the eggs were about 1.4 mm in diameter compared to the 1.6 mm diameter of previous Japanese eel eggs, which suggests they are eggs of a different species. DNA sequencing will be used at Nihon University to determine their species identity. The eel larvae that were collected included 7 pre-feeding stage preleptocephali (Figure 19B,C) collected at 6 different stations that ranged in size from 6.9–8.8 mm. However, those larvae were too large to be Japanese eel preleptocephali, which are 4–5 mm in length. They will also be subjected to DNA sequencing in the laboratory. Serrivomerid leptocephali (8.1–61.4 mm) were the most abundant type of larvae collected, but 8 other species of 6 families were also collected, including 6 quite large leptocephali that were 113–348 mm. One 36.6 mm *Anguilla bicolor pacifica* leptocephalus was also collected (Figure 19E). That is a tropical anguillid species whose spawning area is unknown.

Figure 20. Images related to the upcoming NHK documentary about the YK17-10 cruise and Prof. Tsukamoto's research program on eels, showing images of the R/V YOKOSUKA (A) and the Shinkai 6500 submersible starting dives during the cruise (B,C), as well as a migrating silver eel (D) and a Japanese eel egg from other sources. The header of the Unagi-hakase blog web page is also shown (F) where a daily blog

about the YK17-10 cruise and previous cruises is hosted. Both the online blog and the NHK documentary will provide unique public outreach information about eel research and the activities of JAMSTEC research vessels and underwater observation systems.

4.6 Student education and public outreach

The scientific members of the YK17-10 cruise included 3 graduate students from Nihon University. The eDNA data collected during the cruise will form part of the PhD of one the students. The students also worked with other members of the scientific team to write daily online blogs about the cruise (http://unagi-hakase.jugem.jp/) (Figure 20F). The intense working schedule prevented some days of the blog being posted during the cruise, but each day are being uploaded after the cruise. Photos of the cruise were also posted on the Laboratory of Eel Science Facebook page, which provided further public outreach about the cruise by showing various aspects of the research effort: (https://www.facebook.com/pages/Dr-EEL/1516480831968590?pnref=story).

More importantly 3 participants of the cruise were an NHK camera team that will make a television documentary about the recent eel research of Prof. Tsukamoto that will include video footage from the YK17-10 cruise (Figure 20A-C). This documentary will be aired on the NHK Educational Television Channel (ETV) in September 2017. It will include information about the eel migration studies using PSAT tags that helped guide the present cruise, and will overview the methods and results of the cruise that include detecting Japanese eel eDNA and the history-making observation of an adult anguillid eel underwater for the first time.

4.*7 Future plans*

The results of the present cruise provide new and encouraging results for efforts to observe anguillid eels in their spawning areas. Unfortunately, the anguillid eel recorded by the YKDT observation system was not found until the observations had ended, so that information could not be used to fine-tune the YKDT observation depths, but it can be used for future research. The encouraging results of the eDNA analyses also provide useful information for future research. These positive results including the finding that the eel observed by the YKDT did not appear to show avoidance behavior of the lights of the observation system set the stage for further research. For example, the drifting Una-Cam systems can now be set to a specific depth of about 225 m depths where the anguillid eel was seen at night. Daytime observations from 400–800 m seem to have strong potential for daytime observations based on the eDNA results. Therefore, it is possible that a strategy of deploying some Una-Cams at deep-depths for recording video during the day and others for recording video at shallower depths at night would be a new and more productive observation strategy. In addition, efforts are underway to develop water filtering systems to be installed on

the Una-Cam systems that could collect water-filter samples from the Una-Cam deployments for eDNA analyses to determine if eels are in the locations of the deployments. This combination of a range of new cutting-edge technology has the potential to lead the efforts to learn about the spawning ecology of the Japanese eel to the next level of understanding after the first anguillid eel has been recorded on video during the YK17-10 cruise and many positive eDNA results were obtained in the high-tidal energy patch that was surveyed.

5. Acknowledgements

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